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Ecological Importance of Cross-Feeding of the Intermediate Metabolite 1,2-Propanediol between Bacterial Gut Symbionts

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ABSTRACT Cross-feeding based on the metabolite 1,2-propanediol has been proposed to have an important role in the establishment of trophic interactions among gut symbionts, but its ecological importance has not been empirically established. Here, we show that *in vitro* growth of *Lactobacillus reuteri* (syn. *Limosilactobacillus reuteri*) ATCC PTA 6475 is enhanced through 1,2-propanediol produced by *Bifidobacterium breve* UCC2003 and *Escherichia coli* MG1655 from the metabolization of fucose and rhamnose, respectively. Work with isogenic mutants showed that the trophic interaction is dependent on the *pduCDE* operon in *L. reuteri*, which encodes the ability to use 1,2-propanediol, and the L-fucose permease (*fucP*) gene in *B. breve*, which is required for 1,2-propanediol formation from fucose. Experiments in gnotobiotic mice revealed that, although the *pduCDE* operon bestows a fitness burden on *L. reuteri* ATCC PTA 6475 in the mouse digestive tract, the ecological performance of the strain was enhanced in the presence of *B. breve* UCC2003 and the mucus-degrading species *Bifidobacterium bifidum*. The use of the respective *pduCDE* and *fucP* mutants of *L. reuteri* and *B. breve* in the mouse experiments indicated that the trophic interaction was specifically based on 1,2-propanediol. Overall, our work established the ecological importance of cross-feeding relationships based on 1,2-propanediol for the fitness of a bacterial symbiont in the vertebrate gut.

IMPORTANCE Through experiments in gnotobiotic mice that employed isogenic mutants of bacterial strains that produce (*Bifidobacterium breve*) and utilize (*Lactobacillus reuteri*) 1,2-propanediol, this study provides mechanistic insight into the ecological ramifications of a trophic interaction between gut symbionts. The findings improve our understanding on how cross-feeding influences the competitive fitness of *L. reuteri* in the vertebrate gut and revealed a putative selective force that shaped the evolution of the species. The findings are relevant since they provide a basis to design rational microbial-based strategies to modulate gut ecosystems, which could employ mixtures of bacterial strains that establish trophic interactions or a personalized approach based on the ability of a resident microbiota to provide resources for the incoming microbe.

KEYWORDS 1,2-propanediol, cross-feeding, gut microbiome, *Lactobacillus*, trophic interactions, bifidobacteria, competition, fitness, metabolism, microbial ecology

The gut microbiota is a complex microbial community whose members form interdependent trophic relationships that determine the ecology of bacterial populations and their interplay with the host (1). One such interaction involves the exchange

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of products between microbes, otherwise known as cross-feeding (2). Cross-feeding of nutrients is central to the fermentative degradation of nondigestible dietary fibers and host-derived substrates, such as mucin or human milk oligosaccharides (HMOs) (3–10). For example, glycans released from the partial degradation of HMOs are utilized by other bifidobacterial species (11), and certain bifidobacteria and *Akkermansia muciniphila* liberate glycans from host mucins that are utilized by other inhabitants of the gut (6, 12). Similarly, *Ruminococcus bromii* releases carbohydrates from resistant starch fermentations into the gut environment (13). These interactions ultimately lead to the formation of dynamic metabolic networks essential in the ecology of the gut microbiome and the production of short-chain fatty acids (SCFA), which represent a key ecosystem service that benefits the host (14, 15).

Trophic interactions can also result from the exchange of metabolic end products derived from fermentation processes. Hydrogenotrophic microbes utilize molecular hydrogen produced by several fermentative organisms, a process important to maintain energy flux (16). In addition, cross-feeding based on intermediary metabolites such as lactate, acetate, succinate, and 1,2-propanediol is important for the production of SCFA (5). In the gut, 1,2-propanediol results from the microbial fermentation of deoxyhexose sugars that originate from the hydrolyzation of dietary fiber, fucosylated HMOs, and host mucins, such as rhamnose and fucose (17, 18). Several gut commensal bacteria (i.e., *Bacteroides thetaiotaomicron*, *Roseburia inulinivorans*, *Escherichia coli*, and *Bifidobacterium breve*) can produce 1,2-propanediol from the fermentation of either rhamnose or fucose (3, 5, 17, 18). Other bacteria, such as *Eubacterium hallii* and *Lactobacillus reuteri* (syn. *Limosilactobacillus reuteri* [55]), do not utilize fucose or rhamnose directly but metabolize 1,2-propanediol (19–21) and convert it to propanol and propionate (21, 22). Cross-feeding of 1,2-propanediol between *Eu. hallii* and *Bifidobacterium* species based on metabolism of mucin and HMOs has been studied *in vitro* (3, 4, 6). Although cross-feeding based on 1,2-propanediol is considered important in gut ecosystems (5) and has been shown to confer a fitness advantage *in vivo* for pathogens such as *Salmonella* spp. (23), its ecological importance for bacterial gut symbionts and the underlying mechanisms by which it impacts bacterial performance in the gut have not been empirically established *in vivo* in a tractable experimental model.

In both *Eu. hallii* and *L. reuteri*, 1,2-propanediol is metabolized through the cobalamin-dependent glycerol/diol dehydratase encoded in the *pduCDE* operon, found in the *pdu-cbi-cob-hem* gene (*pdu*) cluster (19–21). The glycerol/diol dehydratase converts 1,2-propanediol to propionaldehyde and further to propanol and propionate and has been shown to increase growth rates for *L. reuteri in vitro* (19, 24). In *L. reuteri*, the *pdu* cluster is observed predominantly in two *L. reuteri* phylogenetic lineages that are dominated by strains from human, herbivore, and chicken origin but is rarer in strains from other hosts such as mice and rats (25). This distribution suggests that the cluster constitutes an adaptation to the characteristics of the gastrointestinal tract of specific hosts (26, 27). In the murine forestomach, which is densely colonized by *L. reuteri* (25), fermentable mono- and disaccharides are in ample supply (28). In contrast, distal portions of the human intestinal tract are characterized by low concentrations of mono- and disaccharides. The *pdu* cluster may therefore constitute a colonization factor in the distal regions of the gut to take advantage of 1,2-propanediol produced by other microbes (19, 25).

Most studies on diol metabolism in *L. reuteri* have focused on the synthesis of the antimicrobial intermediate β-hydroxypropionaldehyde (reuterin) from glycerol, which is also mediated by the *pdu* operon (29). It remains unclear whether *L. reuteri* uses the *pdu* operon to engage in cross-feeding interrelationships with other gut bacterial species that produce 1,2-propanediol in its gastrointestinal habitat and how this interaction contributes to its ecological competitiveness. It was therefore the aim of this study to characterize the ecological importance of 1,2-propanediol-cross-feeding between *L. reuteri* and the 1,2-propanediol producers *Bifidobacterium breve* and *Escherichia coli*, both *in vitro* and in the digestive tracts of mice.

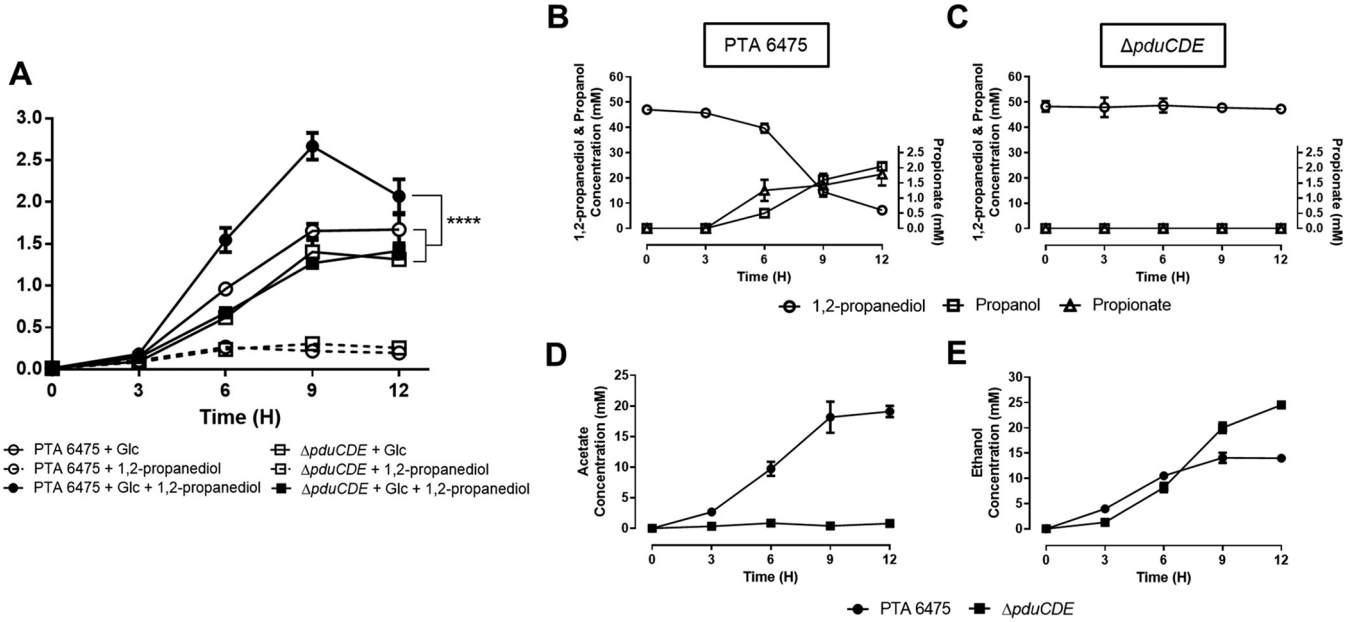

FIG 1 Impact of 1,2-propanediol on growth and metabolism of *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE*. (A) *L. reuteri* strains were grown in half-strength mMRS supplemented with either glucose (Glc; 25 mM), 1,2-propanediol (50 mM), or a mixture of the two. Asterisks indicate a significant difference (two-way ANOVA; $P < 0.001$) in the growth of *L. reuteri* PTA 6475 on glucose plus 1,2-propanediol compared to the other conditions. (B and C) Utilization of 1,2-propanediol and production of propanol and propionate by *L. reuteri* PTA 6475 (B) and *L. reuteri* Δ*pduCDE* (C) during growth on glucose in the presence of 1,2-propanediol. (D and E) Production of acetate (D) and ethanol (E) by the two strains during growth on glucose in the presence of 1,2-propanediol.

RESULTS

1,2-Propanediol enhances growth of *L. reuteri in vitro*. We first sought to confirm the importance of 1,2-propanediol metabolism for *L. reuteri* ATCC PTA 6475 (referred to as *L. reuteri* PTA 6475) growth *in vitro*. The presence of 1,2-propanediol in the medium containing glucose improved growth rates and increased the final cell density of the strain compared to the growth solely on glucose or to growth of a *pduCDE* deletion mutant (referred to as *L. reuteri* Δ*pduCDE*) (Fig. 1A). Neither *L. reuteri* PTA 6475 nor *L. reuteri* Δ*pduCDE* is able to use 1,2-propanediol as the sole carbon source for growth (Fig. 1A). To confirm that the enhanced growth of *L. reuteri* PTA 6475 was due to 1,2-propanediol metabolism, the metabolic end products in the supernatant were measured using high-performance liquid chromatography (HPLC). As shown in Fig. 1B and C, *L. reuteri* PTA 6475, but not *L. reuteri* Δ*pduCDE*, converted 1,2-propanediol to propanol and low concentrations of propionate. For *L. reuteri* PTA 6475, but not *L. reuteri* Δ*pduCDE*, utilization of 1,2-propanediol resulted in acetate production and decreased production of ethanol (Fig. 1D and E; see also Fig. S1A and B in the supplemental material). These findings confirm that *L. reuteri* PTA 6475 is able to disproportionate 1,2-propanediol to propanol and propionate (22). However, 1,2-propanediol is not used as the sole substrate but is cometabolized with glucose. The predominant production of propanol demonstrates that the reducing branch of the propanediol pathway is preferred over the oxidizing branch to regenerate electron acceptors, thus enhancing acetate formation and ATP production and, therefore, growth (30).

Interspecies cross-feeding of 1,2-propanediol enhances growth of *L. reuteri in vitro*. We developed an experimental system to study cross-feeding between *L. reuteri* and gut bacteria that produce 1,2-propanediol. We chose *B. breve* and *E. coli*, which are known to produce 1,2-propanediol from fucose and rhamnose, respectively, which are substrates not utilized by *L. reuteri*. Since growth rates and growth conditions differ among *L. reuteri*, *B. breve*, and *E. coli*, cross-feeding was not studied in cocultures. Instead, *B. breve* and *E. coli* were first grown under their respective optimal conditions and on the specific substrates that result in the production of 1,2-propanediol. Spent

TABLE 1 Media used for *in vitro* cross-feeding experiments

Fermenting strain	Deoxyhexose sugar, concn (mM)	Other carbohydrate, concn (mM)	Abbreviation	Purpose
B. breve UCC2003	NA[a]	Cellobiose, 30	BM (C)	Control for *L. reuteri* growth absent of 1,2-propanediol production
	Fucose, 30	Cellobiose, 30	BM (CF)	For the study of effect of 1,2-propanediol produced from fucose fermentation on *L. reuteri*
B. breve UCC2003-fucP	NA	Cellobiose, 30	B-fucP M (C)	Control for *L. reuteri* growth absent of 1,2-propanediol production
	Fucose, 30	Cellobiose, 30	B-fucP M (CF)	Control for *L. reuteri* growth absent of 1,2-propanediol production
E. coli MG1655	NA	Glucose, 25	EM (G)	Control for *L. reuteri* growth absent of 1,2-propanediol production
	Rhamnose, 30	NA	EM (R)	For the study of effect of 1,2-propanediol produced from rhamnose fermentation on *L. reuteri*

[a]NA, not applicable.

supernatant obtained from these fermentations was then supplemented with glucose and half-strength mMRS (conditioned media; see Materials and Methods) and used for analyzing growth kinetics and metabolite production of the *L. reuteri* strains (Table 1). Conditioned medium from an isogenic mutant of *B. breve* with an insertion mutation in *fucP* (encoding the L-fucose transporter), which was unable to metabolize fucose into 1,2-propanediol, served as a control (Table 1).

B. breve UCC2003 and its *fucP* mutant *B. breve* UCC2003-fucP were grown in a medium containing cellobiose with or without fucose. Fucose does not support the growth of *B. breve* UCC2003 and yet is coutilized with cellobiose to produce 1,2-propanediol. Importantly, neither fucose nor cellobiose is metabolized by *L. reuteri* (20). In the fucose/cellobiose-containing medium, *B. breve* UCC2003 and *B. breve* UCC2003-fucP reached similar cell density after 24 h of growth (see Fig. S2 in the supplemental material). *L. reuteri* PTA 6475 reached a significantly higher optical density at 600 nm (OD_{600}) and showed an elevated growth rate when grown in conditioned medium with the supernatant of *B. breve* UCC2003 grown with fucose and cellobiose compared to *L. reuteri* Δ*pduCDE* or *L. reuteri* PTA 6475 grown in conditioned medium of *B. breve* UCC2003 grown in the absence of fucose (Fig. 2A). The growth advantage of *L. reuteri* PTA 6475 was not observed in conditioned media from the *B. breve* UCC2003-fucP, even if grown in the presence of fucose (Fig. 2B). HPLC analysis confirmed the presence of 1,2-propanediol in the conditioned medium of *B. breve* UCC2003 but not *B. breve* UCC2003-fucP grown with fucose (Fig. S3A) and also showed that enhanced growth of *L. reuteri* PTA 6475 was linked to the conversion of 1,2-propanediol to propanol, which was not detected in *L. reuteri* Δ*pduCDE* cultures (Fig. 2C and D). Propionate, acetate, and ethanol could not be quantified since unknown compounds in the conditioned media interfered with the metabolite analysis. Fucose and cellobiose did not alter the growth kinetics of the *L. reuteri* strains when grown in glucose (Fig. S4A), and they did not serve as growth substrates on their own (Fig. S4B), confirming that the enhanced growth in *L. reuteri* PTA 6475 was not due to a direct effect of residual concentrations of these sugars in the spent supernatant.

1,2-Propanediol results also from the fermentation of rhamnose by *E. coli* MG1655 (Fig. S3B). Cross-feeding experiments revealed that *L. reuteri* PTA 6475 had a higher growth rate and reached a significantly higher cell density when grown in conditioned medium of *E. coli* grown on rhamnose compared to *L. reuteri* Δ*pduCDE* or *L. reuteri* PTA 6475 grown in conditioned medium from *E. coli* that did not contain rhamnose (Fig. 3A). Importantly, growth experiments of *L. reuteri* strains in media with rhamnose with or without glucose confirmed that rhamnose could neither be used as a carbon source nor alter growth (see Fig. S4C and D). Metabolite analysis of fermentations conducted in the conditioned medium of *E. coli* grown on rhamnose revealed that *L. reuteri* PTA 6475, and not *L. reuteri* Δ*pduCDE*, could metabolize 1,2-propanediol produced by *E. coli* and form propanol, propionate, and acetate (Fig. 3B to D; see also Fig. S5). Metabolite interference in the conditioned media did not allow the quantification of ethanol by HPLC.

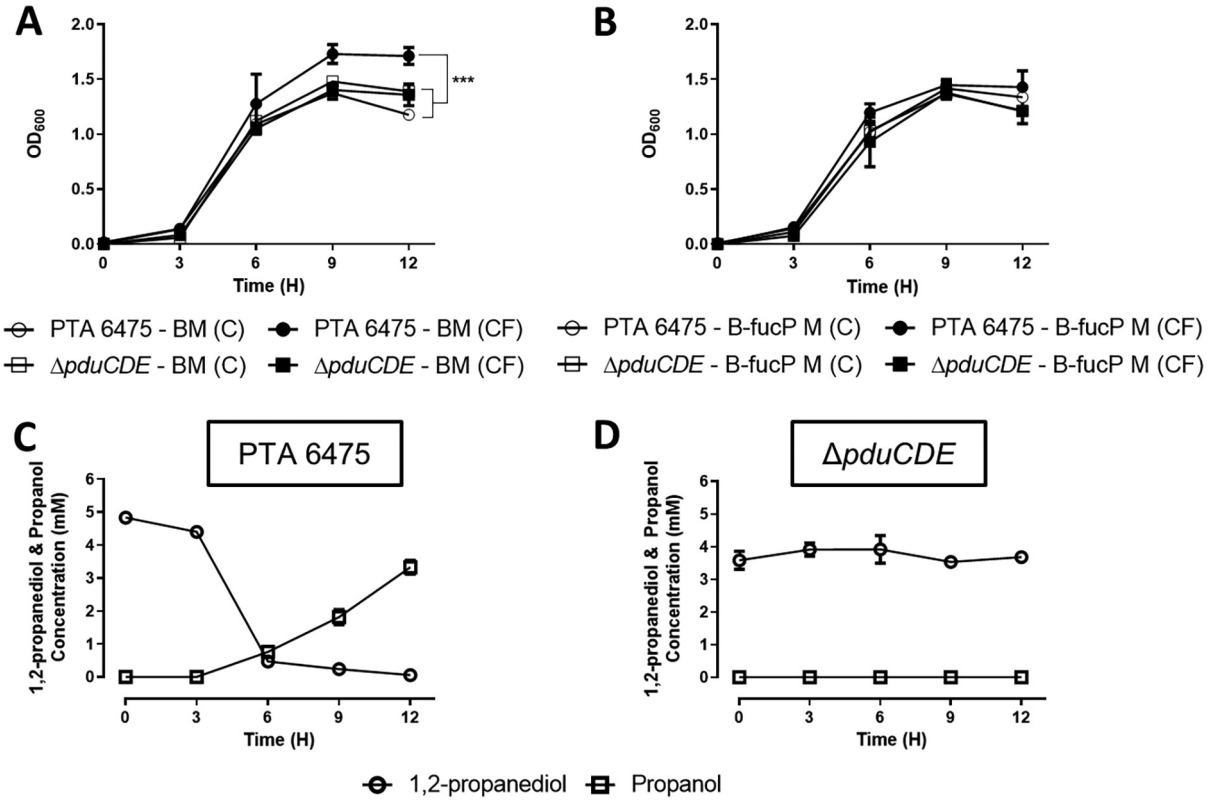

FIG 2 Growth and metabolites of *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE* in conditioned media of *B. breve* UCC2003 and *B. breve* UCC2003-fucP grown with cellobiose alone or with the addition of fucose. (A and B) Growth curves of *L. reuteri* strains in *B. breve* UCC2003 conditioned media (A) and *L. reuteri* in *B. breve* UCC2003-fucP conditioned media (B). Asterisks indicate a significant difference (two-way ANOVA; $P < 0.001$) in growth of *L. reuteri* PTA 6475 grown in *B. breve* UCC2003 conditioned medium that had fermented cellobiose and fucose together compared to growth of *L. reuteri* PTA 6475 in *B. breve* UCC2003 conditioned medium without fucose or to *L. reuteri* Δ*pduCDE* grown in all conditioned media derived from *B. breve* UCC2003. (C and D) Utilization of *B. breve* derived 1,2-propanediol present in the conditioned media and production of propanol in cultures of *L. reuteri* PTA 6475 (C) and *L. reuteri* Δ*pduCDE* (D) grown in the conditioned medium of *B. breve* UCC2003 grown in the presence of fucose. Propionate, acetate, and ethanol concentrations could not be determined due to interference of unknown compounds in the medium. Abbreviations: BM, *B. breve* UCC2003 conditioned media; B-fucP M, *B. breve* UCC2003-fucP conditioned media; (C), preculture fermentations of cellobiose only; (CF), preculture fermentations of cellobiose with added fucose (see Table 1 for more details about the media used in this study).

Together, these findings demonstrate that *L. reuteri* PTA 6475 utilizes 1,2-propanediol produced by *B. breve* and *E. coli* from the fermentation of deoxyhexose sugars as an electron acceptor, enhancing its growth capabilities.

Importance of 1,2-propanediol cross-feeding in the gastrointestinal tract. The ecological relevance of cross-feeding based on 1,2-propanediol in the gastrointestinal tract was investigated with a series of colonization experiments in gnotobiotic mice (Fig. 4; see Tables S1 and S2). Germ-free Swiss-Webster mice (6 to 16 weeks old; male and female) were housed in individually ventilated cages (in groups of two or three). A fat-free diet was used in order to avoid possible confounding effects from the hydrolysis of dietary triglyceride fats, which is a source of glycerol (also utilized via the *pdu* cluster to enhance growth). Fecal cell numbers of colonizing strains were determined by selective plating (see Materials and Methods).

As described earlier, *B. breve* produces 1,2-propanediol from fucose. Host mucins are an intrinsic source of fucose in the gastrointestinal tract, but *B. breve* does not possess glycosidases required for mucin degradation (9). 1,2-Propanediol cross-feeding between *B. breve* and *L. reuteri* was therefore studied in a triple-species associated mouse in the presence of the mucinolytic strain *B. bifidum* PRL2010, which is capable of degrading mucin and releasing fucose without producing 1,2-propanediol (Fig. 4A; see Table S1) (9). We first tested wild-type and mutant *L. reuteri* strains separately. Mice were gavaged with an inoculum that contained *B. bifidum* PRL2010, *B. breve* UCC2003,

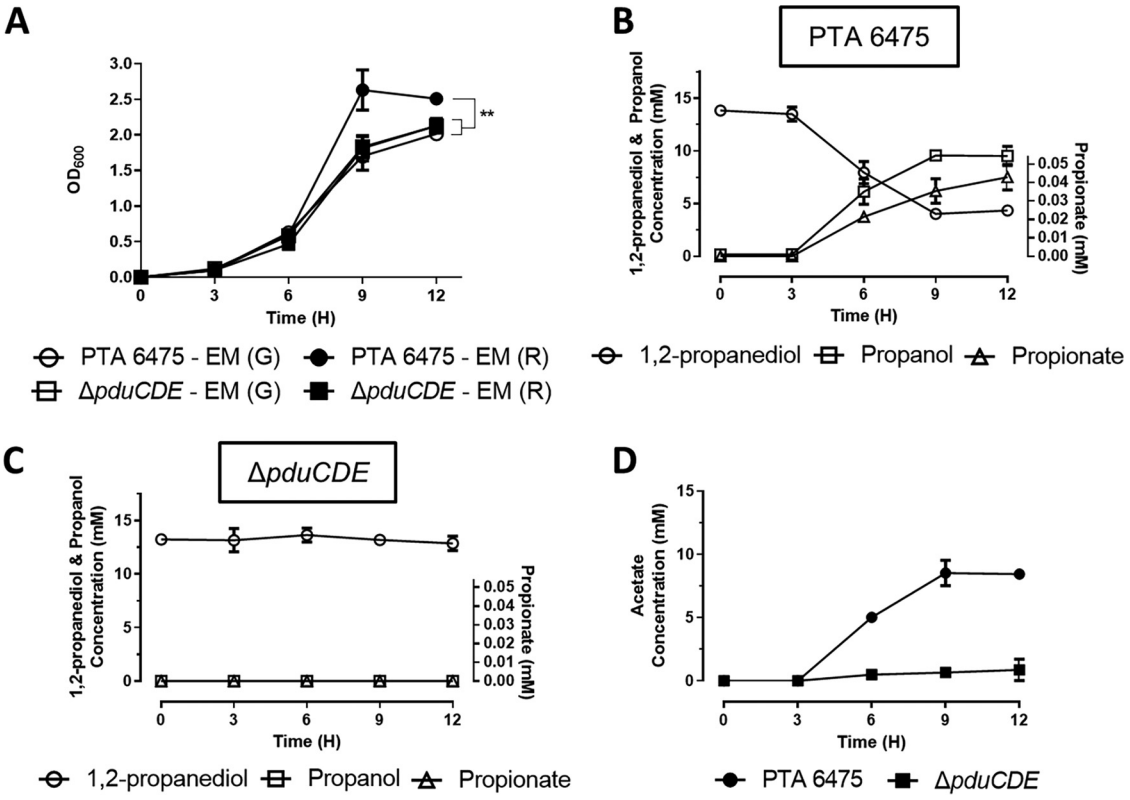

FIG 3 Growth curves and metabolites of *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE* in conditioned media of *E. coli* grown with glucose or rhamnose. (A) Growth of *L. reuteri* strains. Asterisks indicate a significant difference (two-way ANOVA; $P < 0.01$) in growth of *L. reuteri* PTA 6475 grown in conditioned medium from *E. coli* grown on rhamnose compared to conditioned medium from *E. coli* grown on glucose as well as growth of *L. reuteri* Δ*pduCDE* grown in conditioned medium from *E. coli* grown with either glucose or rhamnose. (B and C) Utilization of *E. coli*-derived 1,2-propanediol and production of propanol and propionate by *L. reuteri* PTA 6475 (B) and *L. reuteri* Δ*pduCDE* (C) grown in conditioned medium from *E. coli* grown with rhamnose. (D) Comparison of acetate production by the two strains grown in conditioned medium from *E. coli* grown with rhamnose. Ethanol concentrations could not be determined due to interference of an unknown compound in the medium. Abbreviations: EM, *E. coli* conditioned media; (G), preculture fermentation of glucose only by *E. coli*; (R), preculture fermentation of rhamnose only by *E. coli* (see Table 1 for more details about the media used in the study).

or its *fucP* mutant and also either *L. reuteri* PTA 6475 or *L. reuteri* Δ*pduCDE* (Table S1, inocula A to D). In these experiments, the two *Bifidobacterium* strains formed stable populations of ca. 10^8 to 10^9 CFU/g (see Fig. S6A to H), while the *L. reuteri* strains colonized at ca. 10^6 to 10^8 CFU/g (see Fig. S7A). When colonizing with wild-type *B. breve* UCC2003, *L. reuteri* PTA 6475 formed higher populations than *L. reuteri* Δ*pduCDE* over the duration of the experiment. In contrast, the fecal cell numbers of *L. reuteri* PTA 6475 were consistently lower than those of *L. reuteri* Δ*pduCDE* when colonizing with *B. breve* UCC2003-fucP (but differences did not reach statistical significance due to the high variation between mice) (Fig. S7A). However, normalized ratios (see Materials and Methods) between fecal cell numbers of *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE* were significantly higher in mice colonized with *B. breve* UCC2003 compared to mice colonized with *B. breve* UCC2003-fucP (Fig. 5A). Interestingly, while the ratio between *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE* was greater than 1 when the *L. reuteri* strains were cocolonized with *B. breve* UCC2003, it was substantially lower than 1 when cocolonized with *B. breve* UCC2003-fucP (Fig. 5A). These finding suggests that the *pdu* cluster confers a fitness burden on *L. reuteri* unless 1,2-propanediol is provided, under which the cluster becomes advantageous.

In a parallel set of experiments, we tested the importance of 1,2-propanediol cross-feeding in gnotobiotic mice that were gavaged with *B. bifidum* PRL2010, either *B. breve* UCC2003 or *B. breve* UCC2003-fucP, and both *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE* (wild-type and mutant) in direct competition (Table S1, inocula E and F). In

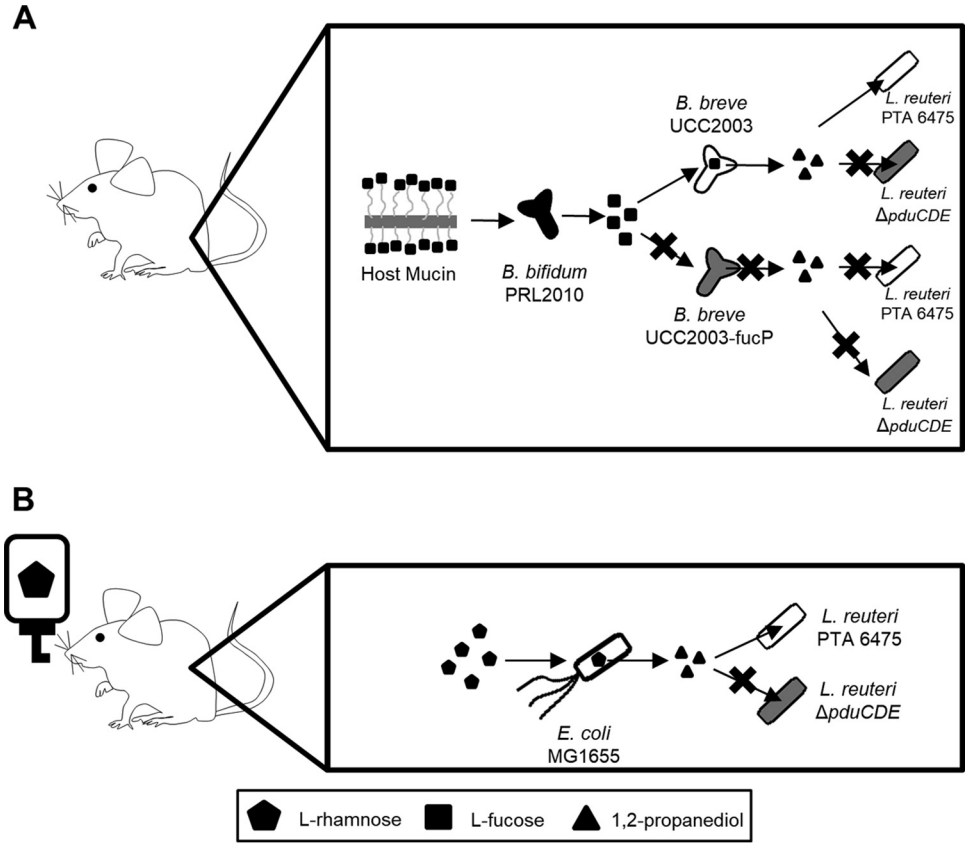

FIG 4 Graphical illustration of hypothesized trophic interactions of 1,2-propanediol in gnotobiotic mice. (A) In triple-species associated gnotobiotic mice (colonized by *B. bifidum*, *B. breve*, and *L. reuteri*), *B. bifidum* liberates fucose from the degradation of host mucin, which is metabolized by *B. breve* UCC2003 producing 1,2-propanediol, that is subsequently utilized by *L. reuteri* PTA 6475. (B) In dual-species (*E. coli* and *L. reuteri*) associated mice whose diet has been supplemented with rhamnose added through the drinking water, *E. coli* metabolizes rhamnose, producing 1,2-propanediol that is subsequently utilized by *L. reuteri* PTA 6475.

agreement with the experiments with only one *L. reuteri* strain, *Bifidobacterium* species formed stable populations that were comparable among groups (ca. 10^8 to 10^9 CFU/g; Fig. S6I to L). *L. reuteri* formed stable populations (ca. 10^6 to 10^8 CFU/g) and, concordant with the experiments using single strains of *L. reuteri*, the cell counts of *L. reuteri* PTA 6475 were higher in mice colonized with *B. breve* UCC2003 than in mice colonized with *B. breve* UCC2003-fucP while counts of *L. reuteri* Δ*pduCDE* were higher in mice colonized with *B. breve* UCC2003-fucP (but the differences were not statistically significant) (Fig. S7B). However, significant differences were observed between the relative proportions of the two *L. reuteri* strains. Specifically, *L. reuteri* PTA 6475 reached significantly higher proportions (>75%) in mice colonized with *B. breve* UCC2003 compared to *B. breve* UCC2003-fucP (Fig. 5B). Interestingly, *L. reuteri* Δ*pduCDE* reached ~75% of the total *Lactobacillus* population in mice colonized with *B. breve* UCC2003-fucP (Fig. 5B), supporting the notion that the *pdu* cluster is a fitness burden to *L. reuteri* unless 1,2-propanediol is provided. Overall, these observations demonstrated that *B. breve* UCC2003 can provide 1,2-propanediol as the result of a trophic chain from the degradation of mucin by *B. bifidum* PRL2010 that facilitates colonization of *L. reuteri* in the gastrointestinal tract (9).

A set of dual-associated gnotobiotic mouse experiments were also conducted to test whether the production of 1,2-propanediol, from the metabolism of rhamnose by *E. coli*, influences the fitness of *L. reuteri* in the gastrointestinal tract. Mice were colonized with *E. coli* and either (i) *L. reuteri* PTA 6475 or *L. reuteri* Δ*pduCDE* alone or (ii) the two strains in competition. Rhamnose was provided to mice in the drinking water

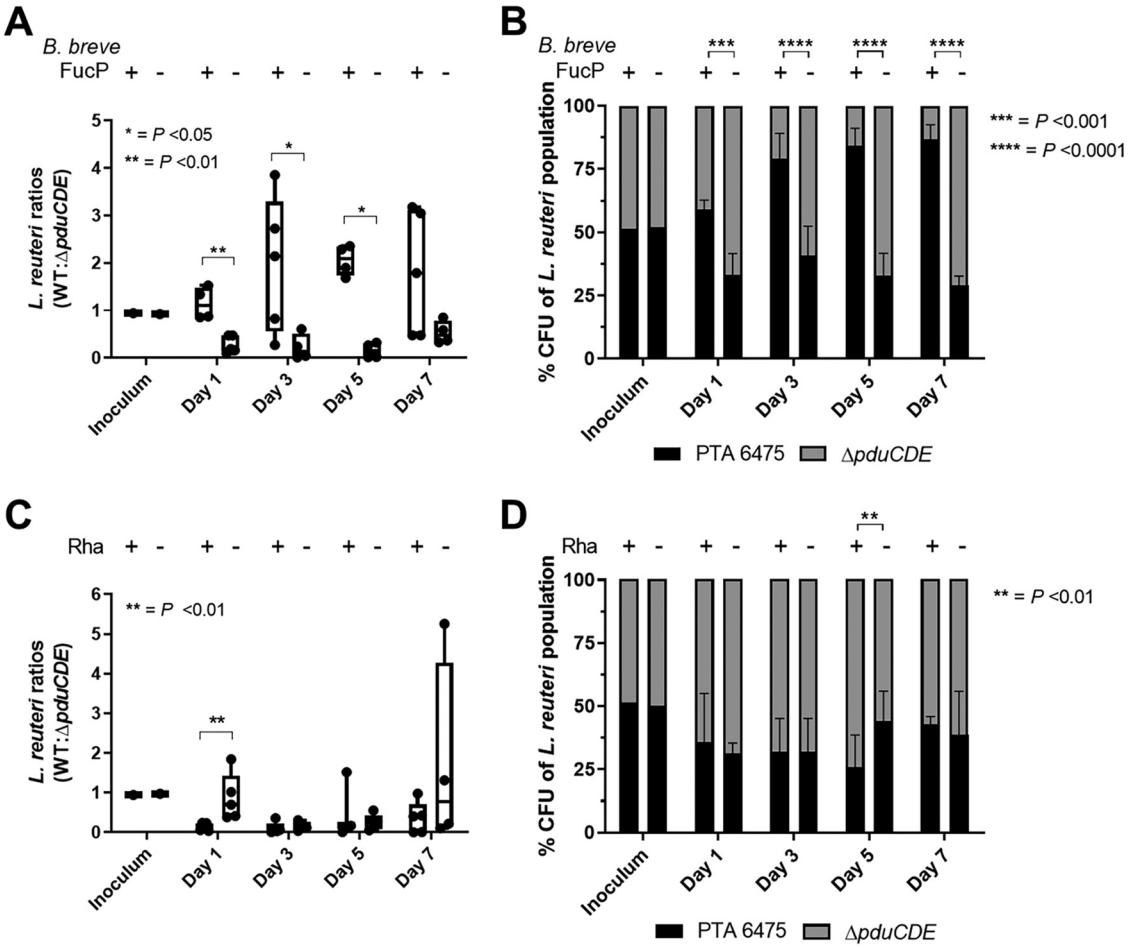

FIG 5 Populations of *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE* in the gastrointestinal tract of triple-species and double-species associated gnotobiotic mice. (A) Normalized ratios between *L. reuteri* PTA 6475 to *L. reuteri* Δ*pduCDE* obtained from *Bifidobacterium-L. reuteri* triple-species associated gnotobiotic mice in which colonization by *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE* was tested separately. A "+" indicates mice colonized with *B. breve* UCC2003, and a "–" indicates mice colonized with *B. breve* UCC2003-fucP. (B) Percent CFU for *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE* as measured in triple-species associated gnotobiotic mice in which the two *L. reuteri* strains were tested in competition. (C) Normalized ratios between *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE* in *E. coli-L. reuteri* double-species associated gnotobiotic mice in which colonization of *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE* was tested separately. (D) Percent CFU for *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE* mutant in double-species associated gnotobiotic mice in which the two *L. reuteri* strains were tested in competition. A "+" indicates the presence of rhamnose (Rha) in the diet, while a "–" indicates absence of rhamnose in the diet. Statistical significance for ratios and percent abundance (% CFU) was determined using a Mann-Whitney test and a Fisher exact test, respectively.

(Fig. 4B; Table S2). Stable populations of *E. coli* were reached in all mice (~10^9 CFU/g), and the provision of rhamnose led to an ~10-fold increase in fecal cell numbers of *E. coli* (Fig. S8). In contrast to the findings with gnotobiotic mice colonized by bifidobacteria, *L. reuteri* Δ*pduCDE* colonized with higher cell numbers and outcompeted *L. reuteri* PTA 6475 under all conditions tested (Fig. 5C and D). This was indicated by ratios of <1 between *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE* in mice colonized with single *L. reuteri* strains (Fig. 5C) and an enrichment of the mutant to ~60% of the total *Lactobacillus* population in competition experiments (Fig. 5D). The latter results resemble those obtained with the triple-species *Bifidobacterium* experiments in mice colonized with *B. breve* UCC2003-fucP (Fig. 5A and B), confirming the fitness burden of the *pdu* cluster.

DISCUSSION

Cross-feeding between members of the gut microbiota based on 1,2-propanediol is considered an important ecological process that shapes the gut microbiome and its metabolism. However, conclusions have so far been derived from *in vitro* experiments

and metagenomic predictions. In this study, we demonstrate that *L. reuteri* engages in trophic interactions with bacteria that are common in the human gut and generate 1,2-propanediol, which is used predominantly to regenerate reduced metabolic cofactors. Using isogenic mutants in both the bacterium that produces 1,2-propanediol and *L. reuteri*, we ensured that this cross-feeding interaction is in fact based on this metabolic intermediate. We further established that the *pduCDE* genes constitute a fitness burden for *L. reuteri* in the gut unless 1,2-propanediol is provided, which makes the cluster ecologically advantageous. Our findings therefore provide insight into both the ecological role of the *pdu* cluster in *L. reuteri* and how it evolved.

Our results demonstrate that *in vitro*, *L. reuteri* can obtain a growth advantage by cross-feeding from 1,2-propanediol derived from the fermentation of fucose and rhamnose by *B. breve* and *E. coli*, respectively. These findings extend previous work showing that *pdu* cluster-containing *L. reuteri* strains grow at a higher rate and to a higher cell yield in the presence of glycerol or 1,2-propanediol (19). In contrast to *L. reuteri* DSM 20016, which produces propanol and propionate in equimolar concentrations through disproportionation when utilizing 1,2-propanendiol as a sole substrate (22), *L. reuteri* PTA 6475 produces propanol in excess over propionate (Fig. 1B) and enhances acetate formation (Fig. 1D) when utilizing 1,2-propanendiol together with glucose. These metabolic patterns suggest that when *L. reuteri* PTA 6475 grows with glucose, 1,2-propanediol functions mainly as an electron acceptor, allowing the conversion of acetyl-phosphate to acetate and to generate an extra ATP (30). Apart from enhancing bacterial growth, 1,2-propanendiol metabolism by *L. reuteri* can therefore potentially contribute to SCFA formation in the gut, which might have health implications as propionate and acetate impact host physiology, e.g., by contributing to gluconeogenesis in the liver, reducing cholesterol, and promoting satiety (5, 18).

Our experiments in gnotobiotic mice provide empirical evidence for the ecological relevance of 1,2-propanendiol cross-feeding for the ecological performance of a gut symbiont in the gastrointestinal tract. The use of the respective *pduCDE* and *fucP* mutants of *L. reuteri* and *B. breve* in the mouse experiments indicated that this trophic interaction was specifically based on 1,2-propanediol produced through the fermentation of fucose. Since *B. breve* cannot degrade mucins and the mouse feed was devoid of fucose, the findings suggest that *L. reuteri* benefits from 1,2-propanediol resulting from interspecies trophic interactions between bifidobacteria, in which *B. bifidum* PRL2010 degrades host mucin and provides fucose for *B. breve* UCC2003 to produce 1,2-propanediol (9). The hydrolysis of mucin is suggested to play a key role in the facilitation of bacterial species in the gut microbiota and has been demonstrated *in vitro* (7, 31, 32) and with bacterial pathogens *in vivo* (e.g., *Salmonella* spp. and *Clostridium difficile*) (23, 33). Our data suggest that it also plays a role in cross-feeding interactions among symbionts or commensals, ultimately conferring a fitness advantage to *L. reuteri* in the murine gut through the provision of 1,2-propanendiol.

Although our experiments demonstrated 1,2-propanediol cross-feeding between bifidobacteria and *L. reuteri*, equivalent findings were not observed in mouse experiments with *L. reuteri* and *E. coli*. This observation can potentially be attributed to a phenomenon called carbon catabolite repression or the "all-or-none" effect in *E. coli*, in which a hierarchy-based regulatory system controls the sequential uptake of carbon sources (34). The mouse diet was highly saturated with glucose (34.4% [wt/wt]) and, *in vitro*, the presence of glucose suppresses the uptake of other carbohydrates in *E. coli* (35), which is in agreement with our finding that *E. coli* MG1655 did not produce 1,2-propanendiol from rhamnose while in the presence of glucose (Fig. S3B). Hence, it is possible that the uptake and metabolism of rhamnose into 1,2-propanediol by *E. coli* was suppressed in the murine gut.

Interestingly, the mouse experiments consistently revealed a fitness burden of the *pduCDE* genes when 1,2-propendiol was absent. Fitness trade-offs are well understood in the evolution of antibiotic resistance in bacteria, where resistance genes are costly and lead to a reduction of growth (36) and are therefore often lost in the absence of antibiotic pressure (37). Our findings indicate that genes that facilitate cross-feeding

interactions are also subjected to fitness trade-offs in that they are only beneficial when the metabolite is provided. Such trade-offs have also been shown in cross-feeding based on the exchange of carbohydrates. *Bacteroides ovatus* possesses an enzyme system dedicated to the digestion of polysaccharides that does not directly benefit itself, but rather cooperative members of the gut microbiota through reciprocal cross-feeding. This enzyme system is energetically unfavorable and in the absence of a reciprocating species, a knockout mutant strain can outcompete the enzyme-encoding wild-type (38). In *L. reuteri*, the fitness burden of the *pdu* genes provides a possible explanation for the evolution of the *pdu* cluster (26). Although likely ancestral to currently known lineages of *L. reuteri*, there is evidence of a deletion of the *pdu* cluster from most rodent strains (25, 26). Growth substrates are abundant in the forestomach and bacteria providing 1,2-propanediol are absent (25, 26), which might have led to loss of the *pdu* cluster since it was evolutionarily advantageous, in agreement with the Black Queen Hypothesis (39). In the human distal gut, 1,2-propanediol is likely provided through other microbiota members, which may explain why the *pdu* cluster is conserved among these strains (26, 27).

Although cross-feeding based on 1,2-propandiol is only one of many trophic interactions that establish interactive networks within the gut microbiota, this study provides important information as it establishes the importance and consequences of cross-feeding for the ecological performance of the involved members. Such knowledge has repercussions for our understanding on the ecological and evolutionary forces that shape gut ecosystems and determine how they function (38, 40–42). In addition, an understanding of mutualistic interactions has important implications since it can be translated into improved microbe-based strategies to modulate gut microbiomes (i.e., probiotics). A challenge encountered in the field of probiotics is that gut ecosystems are homeostatic, resilient to change, and thus difficult to modulate, and most probiotics do not persist or change the resident community (43–46). One solution to this problem is the adoption of an ecological framework for probiotic applications (44). A consideration of the mutualistic and facilitative interactions between community members can be used to design probiotic strain mixtures or personalized probiotic applications with the goal to achieve a more successful long-term persistence, which might be beneficial for certain applications. For example, 1,2-propanediol cross-feeding could be considered in generating probiotic products by pairing *L. reuteri* with *Bifidobacterium* species that release fucose from the degradation of host-derived substrates and convert it into 1,2-propanediol (9, 12). In addition, bifidobacteria are more prevalent in the infant gut (47, 48), and some strains only partly utilize HMOs, releasing fucose, possibly forming an effective synergistic combination with *L. reuteri* (3, 32, 40). Furthermore, cross-feeding of 1,2-propanediol derived from gut symbionts and *S. enterica* serovar Typhimurium has been demonstrated to play a role in promoting pathogen expansion in the gut (23). *L. reuteri* could play a therapeutic role in excluding pathogenic *Salmonella* during gastroenteritis by directly competing for the intermediary metabolite. Overall, this information could not only be used to formulate probiotic mixtures and synbiotic products but could also potentially personalize probiotic applications based on the baseline microbiome (43).

MATERIALS AND METHODS

Bacterial strains and culture conditions. Bacterial strains used in this study are listed in Table 2. *L. reuteri* strains were grown in de Man, Rogosa, and Sharpe (MRS) medium (Difco) in an anaerobic chamber (gas mix of 5% CO_2, 5% H_2, and 90% N_2). *B. bifidum* PRL2010, *B. breve* UCC2003, and *B. breve* UCC2003-fucP were grown anaerobically in MRS medium supplemented with 0.05% L-cysteine. *E. coli* were grown in Luria-Bertani (LB) broth with agitation. All incubations were performed at 37°C.

Evaluation of the impact of 1,2-propanediol on *L. reuteri* ATCC PTA 6475 growth. Overnight cultures of *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE* were inoculated at 1% into 15 ml of half-strength mMRS (49) containing 25 mM glucose alone, 50 mM 1,2-propanediol (Sigma-Aldrich) alone, or 25 mM glucose plus 50 mM 1,2-propanediol. Growth of cell cultures were monitored based on an OD_{600} with a spectrophotometer every 3 h over the span of 12 h. Samples (1 ml) were collected for HPLC analysis every 3 h. All experiments were performed in triplicate at 37°C under anaerobic conditions.

***In vitro* production of 1,2-propanediol and cross-feeding assay development.** Precultures of *B. breve* strains and *E. coli* were prepared as follows. Full-strength mMRS supplemented with 30 mM

TABLE 2 Strains used in this study

Species (strain code)	Origin	Relevant feature(s)	Source and/or reference
Lactobacillus reuteri			
ATCC PTA 6475	Breast milk	1,2-Propanediol utilizer with complete *pdu* cluster	BioGaia (53)
Δ*pduCDE*	Isogenic mutant of PTA 6475	Deletion mutant of glycerol/diol dehydratase genes (*pduCDE*)	53
Escherichia coli			
MG1655	Lab-derived strain	L-Rhamnose utilizer, 1,2-propanediol producer	CGSC[a]
Bifidobacterium bifidum			
PRL2010	Infant stool	Mucin degrader, L-fucose producer	32
Bifidobacterium breve			
UCC2003	Infant stool	L-Fucose utilizer, 1,2-propanediol producer	54
UCC2003-fucP	Isogenic mutant of UCC2003	Insertion mutant of the L-fucose transporter gene (*fucP*)	9

[a]CGSC, Coli Genetic Stock Center.

cellobiose with or without 30 mM L-fucose were inoculated with 1% overnight cultures of *B. breve* UCC2003 or *B. breve* UCC2003-fucP. Full-strength mMRS containing 25 mM glucose or 30 mM L-rhamnose was inoculated with 1% overnight *E. coli* MG1655 cultures. These fermentations were conducted under anaerobic conditions for 24 h at 37°C. Conditioned media were prepared from precultures as follows. Cells were removed from precultures by centrifugation (5,000 × *g* for 10 min), and the supernatant was then collected. The supernatant was supplemented with half-strength mMRS from scratch (50% [wt/vol]; mMRS dry reagents) and glucose (25 mM; dry reagent) to the supernatant. These were further adjusted to pH 6.6 and filter sterilized (0.22 μm), stored at 4°C, and used within 48 h. Conditioned media are described in Table 1. For growth experiments, conditioned media were inoculated with *L. reuteri* strains (1% inoculation). Growth was monitored for 12 h by measuring the OD_{600} with a spectrophotometer, and 1-ml samples for HPLC analysis were collected every 3 h. All experiments were performed in triplicate under anaerobic conditions at 37°C.

Experiments in gnotobiotic mice. All animal experiments were performed with the approval of the Animal Care and Use Committee (ACUC) of the University of Alberta (AUP 00002764). Germ-free Swiss-Webster mice (6 to 16 weeks of age, male and female) were bred and maintained in the Health Sciences Laboratory Animals Services (HSLAS) Facility at the University of Alberta. Mice were randomly selected and moved from a flexible-film isolator and housed in sterile, individually ventilated, positive-pressured biocontainment cages for the duration of the experiments (IsoCage P Biocontainment; Tecniplast). To avoid possible confounding effects of glycerol, which is also utilized by the *pdu* cluster-encoded diol/glycerol dehydratase (50), an irradiated fat-free diet (34.4% glucose and 34.4% cornstarch; Teklad TD.180765) was used in order to minimize possible interference from the hydrolysis of triglyceride fats. After transfer to the biocontainment cages, mice were fed with the new diet for 3 days before colonization with the bacteria.

To study the cross-feeding of 1,2-propanediol in the gastrointestinal tract, groups of mice ($n = 5$), two or three mice per cage, were assigned to receive either *Bifidobacterium-L. reuteri* triple-species mixtures (see Table S1) or *E. coli-L. reuteri* double-species mixtures (see Table S2). To test for cross-feeding of 1,2-propanediol produced from mucin-derived fucose, mice were gavaged with *Bifidobacterium-L. reuteri* triple-species mixtures containing *B. bifidum* PRL2010, either *B. breve* UCC2003 or *B. breve* UCC2003-fucP, and either *L. reuteri* PTA 6475 or *L. reuteri* Δ*pduCDE* (single *L. reuteri* strains), or both strains in competition (Table S1). To test for cross-feeding of dietary rhamnose, we gavaged mice with *E. coli-L. reuteri* double-species mixtures containing *E. coli* and *L. reuteri* PTA 6475 or *L. reuteri* Δ*pduCDE* (single *L. reuteri* strains), or both strains in competition (Table S2). Rhamnose was provided in the drinking water (2% [wt/vol]). Each mouse was gavaged with 200 μl of the corresponding bacterial cell mixtures containing ~10^8 viable cells of each strain. Fecal pellets were collected from individual mice 1, 3, 5, and 7 days after inoculation and plated. Selective plating was used to enumerate bacterial cells in fecal samples as follows: modified Rogosa agar plates were used to quantify *L. reuteri* strains (51). *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE* were differentiated using a reuterin hydrazone detection assay (52). MacConkey agar was used for quantifying *E. coli*. *Bifidobacterium* was selected using *Bifidobacterium* selective iodoacetate mupirocin (BSIM) agar as previously described (48). *B. bifidum* PRL2010 and *B. breve* strains were differentiated based on colony morphology.

Metabolite analysis of postfermentation. 1,2-Propanediol, propanol, propionate, acetate, and ethanol were measured using HPLC. A Bio-Rad Aminex HPX-87H column (300 mm by 7.8 mm) and a refractive index detector were used (HPLC-RI). Samples taken from fermentations were mixed with 70% $HClO_4$ (0.005% [vol/vol]), stored at 4°C overnight to precipitate proteins, centrifuged (18,800 × *g* for 5 min), filtered (0.22 μm), and stored at −20°C before injection into HPLC. Then, 10 μl was injected and eluted with 5 mM H_2SO_4 at a flow rate of 0.4 ml/min at 70°C. 1,2-Propanediol, propanol, propionate, acetate, and ethanol were quantified using external standards.

Statistical analysis. Statistical significance between *L. reuteri* growth curves were determined by two-way analysis of variance (ANOVA) with Bonferroni multiple-comparison test ($\alpha = 0.05$). An unpaired two-tailed Student *t* test was used to analyze significance between *B. breve* growth in mMRS supplemented with cellobiose with or without fucose.

Comparisons between *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE* CFU recovered from fecal samples over the duration of the gnotobiotic mice experiments were performed by using an unpaired two-tailed

Student *t* test. Tests were conducted between *L. reuteri* strains that were associated with (i) *B. breve* UCC2003 or (ii) *B. breve* UCC2003-fucP in the triple-species experiments and *E. coli-L. reuteri* double-species experiments with rhamnose present (iii) or absent (iv).

For the gnotobiotic mouse experiments inoculated with the "single *L. reuteri* strain" mixtures (see Tables S1 and S2), the CFU of *L. reuteri* PTA 6475 and *L. reuteri* Δ*pduCDE* recovered from mouse feces were used to produce normalized ratios. Ratios were generated using the formula (equation 1) below, where a_n is a CFU value for *L. reuteri* PTA 6475 (from a single mouse) used in the comparison, b_n is the CFU value of *L. reuteri* Δ*pduCDE* from each mouse in the group, and n_b is the total population of mice inoculated with the mutant strain used in the experiment.

$$\text{Normalized ratio} = \frac{a_n}{\sum\left(\frac{(b_1 + b_2 + \ldots b_n)}{n_b}\right)} \quad (1)$$

The formula was used to generate sets of ratios for the following comparisons from the "single *L. reuteri* strain" mouse experiments: (i) *B. breve* UCC2003 versus *B. breve* UCC2003-fucP and (ii) *E. coli-L. reuteri* double-associated mice in the presence versus the absence of rhamnose. The statistical significance between the sets of ratios was determined using the Mann-Whitney U test ($P < 0.05$).

The Fisher exact test was used to determine the statistical significance between *L. reuteri* population frequencies from murine groups inoculated with "*L. reuteri* strains in competition" mixtures ($P < 0.05$). This was performed between groups of mice from either (i) *Bifidobacterium-L. reuteri* triple-species associations, including *B. breve* UCC2003 versus *B. breve* UCC2003-fucP, or (ii) *E. coli-L. reuteri* double-species associations with a murine diet supplemented with rhamnose versus without rhamnose. Statistical analyses were performed using GraphPad Prism 6.07.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.9 MB.

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C.C.C. designed and conducted the experiments, collected and analyzed data, and wrote the manuscript. R.M.D. contributed to conceiving the project, the development of experimental approaches, and supervision, provided intellectual leadership, and wrote the manuscript. X.L. contributed to experimental designs and edited the manuscript. M.E.P.-M. contributed to the design of animal experiments and contributed to the manuscript through editing and the generation of figures. S.T. was responsible for animal husbandry and oversaw animal experiments. J.-H.O. and J.-P.V.P. generated the mutant *L. reuteri* ATCC PTA 6475 Δ*pduCDE* strains and contributed to manuscript editing. F.L. contributed to supervision and manuscript editing. D.V.S. provided the *Bifidobacterium bifidum* PRL2010, *Bifidobacterium breve* UCC2003, *Bifidobacterium breve* UCC2003-fucP strains; contributed to the design of the animal experiments; and gave technical and conceptual advice. M.G.G. oversaw analytical analysis and data interpretation and contributed to supervision and manuscript editing. J.W. conceived the project, contributed to the conceptualization of the experiments, supervised data analysis and interpretation, and wrote the manuscript.

ADDENDUM IN PROOF

During the proof stage, a taxonomic note that proposed the reclassification of the genus *Lactobacillus* into 25 genera was published (55). According to that proposal, what is described in the present article as *Lactobacillus reuteri* is now *Limosilactobacillus reuteri*.

REFERENCES

1. Thursby E, Juge N. 2017. Introduction to the human gut microbiota. Biochem J 474:1823–1836. https://doi.org/10.1042/BCJ20160510.
2. D'Souza G, Shitut S, Preussger D, Yousif G, Waschina S, Kost C. 2018. Ecology and evolution of metabolic cross-feeding interactions in bacteria. Nat Prod Rep 35:455–488. https://doi.org/10.1039/c8np00009c.
3. Bunesova V, Lacroix C, Schwab C. 2018. Mucin cross-feeding of infant

bifidobacteria and *Eubacterium hallii*. Microb Ecol 75:228–238. https://doi.org/10.1007/s00248-017-1037-4.
4. Schwab C, Ruscheweyh H, Bunesova V, Pham VT, Beerenwinkel N, Lacroix C. 2017. Trophic interactions of infant bifidobacteria and *Eubacterium hallii* during L-fucose and fucosyllactose degradation. Front Microbiol 8:95. https://doi.org/10.3389/fmicb.2017.00095.
5. Louis P, Flint HJ. 2017. Formation of propionate and butyrate by the human colonic microbiota. Environ Microbiol 19:29–41. https://doi.org/10.1111/1462-2920.13589.
6. Belzer C, Chia LW, Aalvink S, Chamlagain B, Piironen V, Knol J, de Vos WM. 2017. Microbial metabolic networks at the mucus layer lead to diet-independent butyrate and vitamin B_{12} production by intestinal symbionts. mBio 8:e00770-17. https://doi.org/10.1128/mBio.00770-17.
7. Martens EC, Chiang HC, Gordon JI. 2008. Mucosal glycan foraging enhances fitness and transmission of a saccharolytic human gut bacterial symbiont. Cell Host Microbe 4:447–457. https://doi.org/10.1016/j.chom.2008.09.007.
8. Egan M, O'Connell Motherway M, Ventura M, van Sinderen D. 2014. Metabolism of sialic acid by *Bifidobacterium breve* UCC2003. Appl Environ Microbiol 80:4414–4426. https://doi.org/10.1128/AEM.01114-14.
9. Egan M, O'Connell Motherway M, Kilcoyne M, Kane M, Joshi L, Ventura M, van Sinderen D. 2014. Cross-feeding by *Bifidobacterium breve* UCC2003 during cocultivation with *Bifidobacterium bifidum* PRL2010 in a mucin-based medium. BMC Microbiol 14:282. https://doi.org/10.1186/s12866-014-0282-7.
10. Duranti S, Lugli GA, Milani C, James K, Mancabelli L, Turroni F, Alessandri G, Mangifesta M, Mancino W, Ossiprandi MC, Iori A, Rota C, Gargano G, Bernasconi S, Di Pierro F, van Sinderen D, Ventura M. 2019. *Bifidobacterium bifidum* and the infant gut microbiota: an intriguing case of microbe-host co-evolution. Environ Microbiol 21:3686–3695. https://doi.org/10.1111/1462-2920.14705.
11. Gotoh A, Katoh T, Sakanaka M, Ling Y, Yamada C, Asakuma S, Urashima T, Tomabechi Y, Katayama-Ikegami A, Kurihara S, Yamamoto K, Harata G, He F, Hirose J, Kitaoka M, Okuda S, Katayama T. 2018. Sharing of human milk oligosaccharides degradants within bifidobacterial communities in faecal cultures supplemented with *Bifidobacterium bifidum*. Sci Rep 8:13958. https://doi.org/10.1038/s41598-018-32080-3.
12. Tailford LE, Crost EH, Kavanaugh D, Juge N. 2015. Mucin glycan foraging in the human gut microbiome. Front Genet 6:81. https://doi.org/10.3389/fgene.2015.00081.
13. Ze X, Duncan SH, Louis P, Flint HJ. 2012. *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. ISME J 6:1535–1543. https://doi.org/10.1038/ismej.2012.4.
14. Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. 2012. Microbial degradation of complex carbohydrates in the gut. Gut Microbes 3:289–306. https://doi.org/10.4161/gmic.19897.
15. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. 2016. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell 165:1332–1345. https://doi.org/10.1016/j.cell.2016.05.041.
16. Smith NW, Shorten PR, Altermann EH, Roy NC, McNabb WC. 2019. Hydrogen cross-feeders of the human gastrointestinal tract. Gut Microbes 10:270–288. https://doi.org/10.1080/19490976.2018.1546522.
17. Saxena RK, Anand P, Saran S, Isar J, Agarwal L. 2010. Microbial production and applications of 1,2-propanediol. Indian J Microbiol 50:2–11. https://doi.org/10.1007/s12088-010-0017-x.
18. Reichardt N, Duncan SH, Young P, Belenguer A, McWilliam Leitch C, Scott KP, Flint HJ, Louis P. 2014. Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. ISME J 8:1323–1335. https://doi.org/10.1038/ismej.2014.14.
19. Rattanaprasert M, Roos S, Hutkins RW, Walter J. 2014. Quantitative evaluation of synbiotic strategies to improve persistence and metabolic activity of *Lactobacillus reuteri* DSM 17938 in the human gastrointestinal tract. J Funct Foods 10:85–94. https://doi.org/10.1016/j.jff.2014.05.017.
20. Schwab C, Gänzle M. 2011. Lactic acid bacteria fermentation of human milk oligosaccharide components, human milk oligosaccharides and galactooligosaccharides. FEMS Microbiol Lett 315:141–148. https://doi.org/10.1111/j.1574-6968.2010.02185.x.
21. Engels C, Ruscheweyh H, Beerenwinkel N, Lacroix C, Schwab C. 2016. The common gut microbe *Eubacterium hallii* also contributes to intestinal propionate formation. Front Microbiol 7:713. https://doi.org/10.3389/fmicb.2016.00713.
22. Sriramulu DD, Liang M, Hernandez-Romero D, Raux-Deery E, Lunsdorf H, Parsons JB, Warren MJ, Prentice MB. *Lactobacillus reuteri* DSM 20016 produces cobalamin-dependent diol dehydratase in metabolosomes and metabolizes 1,2-propanediol by disproportionation. J Bacteriol 190:4559–4567. https://doi.org/10.1128/JB.01535-07.
23. Faber F, Thiennimitr P, Spiga L, Byndloss MX, Litvak Y, Lawhon S, Andrews-Polymenis HL, Winter SE, Bäumler AJ. 2017. Respiration of microbiota-derived 1,2-propanediol drives *Salmonella* expansion during colitis. PLoS Pathog 13:e1006129. https://doi.org/10.1371/journal.ppat.1006129.
24. Chen L, Hatti-Kaul R. 2017. Exploring *Lactobacillus reuteri* DSM20016 as a biocatalyst for transformation of longer chain 1,2-diols: limits with microcompartment. PLoS One 12:e0185734. https://doi.org/10.1371/journal.pone.0185734.
25. Walter J, Britton RA, Roos S. 2011. Host-microbial symbiosis in the vertebrate gastrointestinal tract and the *Lactobacillus reuteri* paradigm. Proc Natl Acad Sci U S A 108:4645–4652. https://doi.org/10.1073/pnas.1000099107.
26. Frese SA, Benson AK, Tannock GW, Loach DM, Kim J, Zhang M, Oh PL, Heng NCK, Patil PB, Juge N, Mackenzie DA, Pearson BM, Lapidus A, Dalin E, Tice H, Goltsman E, Land M, Hauser L, Ivanova N, Kyrpides NC, Walter J. 2011. The evolution of host specialization in the vertebrate gut symbiont *Lactobacillus reuteri*. PLoS Genet 7:e1001314. https://doi.org/10.1371/journal.pgen.1001314.
27. Oh PL, Benson AK, Peterson DA, Patil PB, Moriyama EN, Roos S, Walter J. 2010. Diversification of the gut symbiont *Lactobacillus reuteri* as a result of host-driven evolution. ISME J 4:377–387. https://doi.org/10.1038/ismej.2009.123.
28. Tannock GW, Wilson CM, Loach D, Cook GM, Eason J, O'Toole PW, Holtrop G, Lawley B. 2012. Resource partitioning in relation to cohabitation of *Lactobacillus* species in the mouse forestomach. ISME J 6:927–938. https://doi.org/10.1038/ismej.2011.161.
29. Cleusix V, Lacroix C, Vollenweider S, Duboux M, Le Blay G. 2007. Inhibitory activity spectrum of reuterin produced by *Lactobacillus reuteri* against intestinal bacteria. BMC Microbiol 7:101. https://doi.org/10.1186/1471-2180-7-101.
30. Gänzle MG. 2015. Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. Curr Opin Food Sci 2:106–117. https://doi.org/10.1016/j.cofs.2015.03.001.
31. Turroni F, Milani C, Duranti S, Mahony J, van Sinderen D, Ventura M. 2018. Glycan utilization and cross-feeding activities by bifidobacteria. Trends Microbiol 26:339–350. https://doi.org/10.1016/j.tim.2017.10.001.
32. Turroni F, Bottacini F, Foroni E, Mulder I, Kim J, Zomer A, Sánchez B, Bidossi A, Ferrarini A, Giubellini V, Delledonne M, Henrissat B, Coutinho P, Oggioni M, Fitzgerald GF, Mills D, Margolles A, Kelly D, van Sinderen D, Ventura M, Klaenhammer TR. 2010. Genome analysis of *Bifidobacterium bifidum* PRL2010 reveals metabolic pathways for host-derived glycan foraging. Proc Natl Acad Sci U S A 107:19514–19519. https://doi.org/10.1073/pnas.1011100107.
33. Ng KM, Ferreyra JA, Higginbottom SK, Lynch JB, Kashyap PC, Gopinath S, Naidu N, Choudhury B, Weimer BC, Monack DM, Sonnenburg JL. 2013. Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. Nature 502:96–99. https://doi.org/10.1038/nature12503.
34. Kremling A, Geiselmann J, Ropers D, de Jong H. 2015. Understanding carbon catabolite repression in *Escherichia coli* using quantitative models. Trends Microbiol 23:99–109. https://doi.org/10.1016/j.tim.2014.11.002.
35. Aidelberg G, Towbin BD, Rothschild D, Dekel E, Bren A, Alon U. 2014. Hierarchy of non-glucose sugars in *Escherichia coli*. BMC Syst Biol 8:133. https://doi.org/10.1186/s12918-014-0133-z.
36. Basra P, Alsaadi A, Bernal-Astrain G, O'Sullivan ML, Hazlett B, Clarke LM, Schoenrock A, Pitre S, Wong A. 2018. Fitness trade-offs of antibiotic resistance in extraintestinal pathogenic *Escherichia coli*. Genome Biol Evol 10:667–679. https://doi.org/10.1093/gbe/evy030.
37. Andersson DI, Hughes D. 2010. Antibiotic resistance and its cost: is it possible to reverse resistance? Nat Rev Microbiol 8:260–271. https://doi.org/10.1038/nrmicro2319.
38. Rakoff-Nahoum S, Foster KR, Comstock LE. 2016. The evolution of cooperation within the gut microbiota. Nature 533:255–259. https://doi.org/10.1038/nature17626.
39. Smith NW, Shorten PR, Altermann E, Roy NC, McNabb WC. 2019. The classification and evolution of bacterial cross-feeding. Front Ecol Evol 7:153. https://doi.org/10.3389/fevo.2019.00153.
40. O'Connell Motherway M, O'Brien F, O'Driscoll T, Casey PG, Shanahan F,

van Sinderen D. 2018. Carbohydrate syntrophy enhances the establishment of *Bifidobacterium breve* UCC2003 in the neonatal gut. Sci Rep 8:10627. https://doi.org/10.1038/s41598-018-29034-0.
41. Coyte KZ, Rakoff-Nahoum S. 2019. Understanding competition and cooperation within the mammalian gut microbiome. Curr Biol 29:R538–R544. https://doi.org/10.1016/j.cub.2019.04.017.
42. Rakoff-Nahoum S, Coyne M, Comstock L. 2014. An ecological network of polysaccharide utilization among human intestinal symbionts. Curr Biol 24:40–49. https://doi.org/10.1016/j.cub.2013.10.077.
43. Maldonado-Gómez MX, Martínez I, Bottacini F, O'Callaghan A, Ventura M, van Sinderen D, Hillmann B, Vangay P, Knights D, Hutkins RW, Walter J. 2016. Stable engraftment of *Bifidobacterium longum* AH1206 in the human gut depends on individualized features of the resident microbiome. Cell Host Microbe 20:515–526. https://doi.org/10.1016/j.chom.2016.09.001.
44. Walter J, Maldonado-Gómez MX, Martínez I. 2018. To engraft or not to engraft: an ecological framework for gut microbiome modulation with live microbes. Curr Opin Biotechnol 49:129–139. https://doi.org/10.1016/j.copbio.2017.08.008.
45. Khalesi S, Bellissimo N, Vandelanotte C, Williams S, Stanley D, Irwin C. 2019. A review of probiotic supplementation in healthy adults: helpful or hype? Eur J Clin Nutr 73:24–37. https://doi.org/10.1038/s41430-018-0135-9.
46. Zmora N, Zilberman-Schapira G, Suez J, Mor U, Dori-Bachash M, Bashiardes S, Kotler E, Zur M, Regev-Lehavi D, Brik RB, Federici Sw, Cohen Y, Linevsky R, Rothschild D, Moor AE, Ben-Moshe S, Harmelin A, Itzkovitz S, Maharshak N, Shibolet O, Shapiro H, Pevsner-Fischer M, Sharon I, Halpern Z, Segal E, Elinav E. 2018. Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. Cell 174:1388–1405.e21. https://doi.org/10.1016/j.cell.2018.08.041.
47. Ferretti P, Pasolli E, Tett A, Asnicar F, Gorfer V, Fedi S, Armanini F, Truong DT, Manara S, Zolfo M, Beghini F, Bertorelli R, De Sanctis V, Bariletti I, Canto R, Clementi R, Cologna M, Crifò T, Cusumano G, Gottardi S, Innamorati C, Masè C, Postai D, Savoi D, Duranti S, Lugli GA, Mancabelli L, Turroni F, Ferrario C, Milani C, Mangifesta M, Anzalone R, Viappiani A, Yassour M, Vlamakis H, Xavier R, Collado CM, Koren O, Tateo S, Soffiati M, Pedrotti A, Ventura M, Huttenhower C, Bork P, Segata N. 2018. Mother-to-Infant microbial transmission from different body sites shapes the developing infant gut microbiome. Cell Host Microbe 24:133–145.e5. https://doi.org/10.1016/j.chom.2018.06.005.
48. Lewis ZT, Totten SM, Smilowitz JT, Popovic M, Parker E, Lemay DG, Tassell ML, Miller MJ, Jin Y, German JB, Lebrilla CB, Mills DA. 2015. Maternal fucosyltransferase 2 status affects the gut bifidobacterial communities of breastfed infants. Microbiome 3:13. https://doi.org/10.1186/s40168-015-0071-z.
49. Stolz P, Vogel RF, Hammes WP. 1995. Utilization of electron acceptors by *lactobacilli* isolated from sourdough. Z Lebensm Unters Forch 201:402–410. https://doi.org/10.1007/BF01192742.
50. Talarico TL, Axelsson LT, Novotny J, Fiuzat M, Dobrogosz WJ. 1990. Utilization of glycerol as a hydrogen acceptor by *Lactobacillus reuteri*: purification of 1,3-propanediol:NAD^+ oxidoreductase. Appl Environ Microbiol 56:943–948. https://doi.org/10.1128/AEM.56.4.943-948.1990.
51. Duar RM, Frese SA, Lin XB, Fernando SC, Burkey TE, Tasseva G, Peterson DA, Blom J, Wenzel CQ, Szymanski CM, Walter J. 2017. Experimental evaluation of host adaptation of *Lactobacillus reuteri* to different vertebrate species. Appl Environ Microbiol 83:e00132-17. https://doi.org/10.1128/AEM.00132-17.
52. Rosander A, Connolly E, Roos S. 2008. Removal of antibiotic resistance gene-carrying plasmids from *Lactobacillus reuteri* ATCC 55730 and characterization of the resulting daughter strain, *L. reuteri* DSM 17938. Appl Environ Microbiol 74:6032–6040. https://doi.org/10.1128/AEM.00991-08.
53. Zhang S, Oh J, Alexander LM, Özçam M, van Pijkeren J. 2018. D-Alanyl-D-alanine ligase as a broad-host-range counterselection marker in vancomycin-resistant lactic acid bacteria. J Bacteriol 200:e00607-17. https://doi.org/10.1128/JB.00607-17.
54. Mazé A, O'Connell-Motherway M, Fitzgerald GF, Deutscher J, van Sinderen D. 2007. Identification and characterization of a fructose phosphotransferase system in *Bifidobacterium breve* UCC2003. Appl Environ Microbiol 73:545–553. https://doi.org/10.1128/AEM.01496-06.
55. Zheng J, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P, O'Toole PW, Pot B, Vandamme P, Walter J, Watanabe K, Wuyts S, Felis GE, Gänzle MG, Lebeer S. 15 April 2020. A taxonomic note on the genus *Lactobacillus*: description of 23 novel genera, emended description of the genus *Lactobacillus Beijerinck* 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. Int J Syst Evol Microbiol https://doi.org/10.1099/ijsem.0.004107.